Exploring the function and behaviour of Natural Populations of Coral Reef Microbes

JESSICA TOUT
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Certificate of Original Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Jessica Tout
1/02/2016

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Publications

Peer reviewed journal articles arising directly from this thesis:

Chapter 1

Tout J, Jeffries TC, Webster NS, Stocker R, Ralph PJ, Seymour JR (2014). Variability in Microbial Community Composition and Function Between Different Niches Within a Coral Reef. Microbial Ecology. 67 (3): 540-552

Chapter 2

Tout J, Jeffries TC, Petrou K, Tyson GW, Webster NS, Garren M, Stocker R, Ralph PJ, Seymour JR (2015). Chemotaxis by Natural Populations of Coral Reef Bacteria. ISME J doi: 10.1038/ismej.2014.261

Chapter 4

Tout J, Siboni N, Messer LF, Garren M, Stocker R, Webster NS, Ralph PJ and Seymour JR (2015). Increased seawater temperature increases the abundance and alters the structure of natural *Vibrio* populations associated with the coral Pocillopora damicornis. *Front. Microbiol.* 6:432. doi: 10.3389/fmicb.2015.00432

Chapter 5

Garren M, Son K, Raina JB, Rusconi R, Menolascina F, Shapiro OH, **Tout J**, Bourne DG, Seymour JR, Stocker R (2013). A bacterial pathogen uses dimethylsulfoniopropionate as a cue to target heat-stressed corals. ISME J 1-9, doi:10.1038/ismej.2013.210

See Appendix

Garren M, Son K, **Tout J,** Seymour JR, Stocker R (2015). Temperature-induced behavioural switches in a bacterial coral pathogen. ISME J 1-10, doi:10.1038/ismej.2015.216

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responding to each chemoattractant were normalised by multiplying by the cell abundance retrieved from FCM (Figure 1) (Dennis et al., 2013). The microbial community identified in the FSW control is representative of organisms that swam into this treatment as a consequence of random motility, rather than chemotaxis, due to the lack of any chemical gradient. As such, this sample provides an overview of the motile, but not necessarily chemotactic proportion of the community. For more detail on unique taxa responding to individual chemoattractants, see Supplementary Figure S3.

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Supplementary Figure S1.1 starting from top left, "x" marks the area from which water was collected from. 1) The sandy substrate niche – following water collection in this niche, the brick was placed above where water was collected for a separate experiment, 2) the lagoon-coral niche: *Acropora palifera*, 3) the open water niche, water was collected prior to where this cylinder was placed for a separate experiment, and 4) the reef crest-coral niche: *Acropora aspera.*

Supplementary Figure S1.2 Relative % of SEED matches to Archaea in each seawater niche metagenome: sandy substrate, lagoon-coral, reef crest-coral and open water (ordered according to frequency of occurrence in each niche).

Supplementary Figure S1.3 Relative % of SEED matches to Viruses in each seawater niche metagenome: sandy substrate, lagoon-coral, reef crest-coral and open water (ordered according to frequency of occurrence in each niche).

Supplementary Figure S1.4 Relative % of SEED matches to Eukaryota greater than 0.05% in each seawater niche metagenome: sandy substrate, lagoon-coral, reef crest-coral and open water (ordered according to frequency of occurrence in each niche).

Supplementary figure S2.1 Map showing location of a) Heron Island on the Great Barrier Reef, Australia indicated by a red dot, and b) sample sites on Heron Island, the Great Barrier Reef where a) triangles depict sites where water was collected in July 2010 for laboratory experiments using syringe assays: blue depicts the non-coral associated site outside of the reef crest and red shows the coral-associated site on the surface of *Pocillopora damicornis* within the reef crest and b) circles represent microenvironments where *In Situ* Chemotaxis Assays (ISCAs) were deployed in July 2011 to collect both cell counts for flow cytometry and DNA for 16S rRNA amplicon pyrosequencing.

Supplementary figure S2.2 Photograph depicting a ISCA prior to being fully inserted in between the branches of *Acropora palifera* in the lagoon, Heron Island.

Supplementary figure S2.3 Taxonomic identity (genus level) of the bacterial community inhabiting seawater collected from the surface of the coral species *Acropora aspera.* Relative representation in the metagenome was calculated by normalizing to total number of sequences. Taxonomy was assigned by using BLASTn to compare sequences to the Greengenes database in MG RAST. The composition of this bulk, background community differs substantially to the

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Supplementary figure S2.4: a) Taxonomic identity of chemotactic bacteria determined using 16S rRNA tag pyrosequencing (not normalised to cell counts as represented in Fig. 3. Hits were generated by comparing the sequences with BLASTn to the Greengenes database in QIIME. Chao1 diversity estimates revealed that carbohydrates had the highest diversity of all the chemoattractants at 236, followed by amino acids at 158.4, DSMP had a chao1 of 130, while ammonium chloride had a diversity of 125. b) Taxonomic identity of chemotactic bacteria unique to each chemoattractant (ie found in only that chemoattractant) as seen in Fig. 2.3 and Fig. 2.4a.

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abundance compared to the coral metagenomes and overall contributed to a cumulative 5.7% of dissimilarity between the metagenomes. Copiotrophic genera such as *Alteromonas, Mycobacterium* and *Vibrio* were shown to be discriminating genera in the coral metagenomes as they had a higher average abundance compared to the non-coral metagenome and contributed up to 3.6% of the dissimilarity between metagenomes.

Supplementary Table S1.2: STAMP output represented as a table rather than the STAMP graph due to the large size where Fisher's exact test was used to statistically test for significant differences in the relative representation of microbial genera between the lagoon-coral community and open water community.

Supplementary Table S1.3: STAMP output represented as a table rather than the STAMP graph due to the large size where Fisher's exact test was used to statistically test for significant differences in the relative representation of microbial genera between the lagoon-coral community and the sandy substrate water community.

Supplementary Table S 1.4 PRIMER table showing SIMPER analysis of metabolic categories between coral (lagoon-coral and reef crest-coral) and non-coral (open water and sandy substrate) metagenomes with an average dissimilarity of 1.55. Less abundant metabolic functions including genes associated with Phage function and motility and chemotaxis were found to be discriminating functions of the coral niches (lagoon-coral and reef crest-coral) and overall contributed to a cumulative of 17.7% of the dissimilarity between metagenomes.

Supplementary table S2.1: Two-way fixed factor ANOVA to determine chemotactic response using a modified capillary assay to DMSP at three concentrations in two water types (coral reef and non-coral reef). Table relates to data presented in Figure 2.1a i-ii.

Supplementary table S2.2: Three-way fixed factor ANOVA of the effects of water type (coral reef and non-coral reef), amino acid, and concentration on the chemotactic response of bacteria using a modified capillary assay. Table relates to data presented in Figure 2.1b i-ii.

Supplementary table S2.3: Three-way fixed factor ANOVA on the effects of water type (coral reef and non-coral reef), carbohydrate, and concentration on the chemotactic response of bacteria using a modified capillary assay. Table relates to data presented in Figure 2.1c i-ii.

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dissimilarity of 7.19%. Genes associated with cell motility contributed to 46.09% of the differences between the coral-associated and non-coral associated samples.

Supplementary Table S2.10: PRIMER table showing SIMPER analysis of metabolic categories between coral (lagoon-coral and reef crest-coral) and non-coral (open water and sandy substrate) metagenomes with an average dissimilarity of 13.72. Genes associated with bacterial chemotaxis contributed to 21.39% of the differences between the coral-associated and non-coral associated samples.

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Supplementary Table S4.4: PRIMER table showing SIMPER analysis between the t_{final} Heat Stress and t_0 treatments with an average dissimilarity of 56.02%.

Supplementary Table S4.5: A 1-way ANOVA was used to determine the significant differences between the abundances of vibrios per cm^2 of *P. damicornis* for each treatment.

Supplementary Table S4.6: A 1-way ANOVA was used to determine the significant differences between the abundances of *V. coralliilyticus* per cm^2 of *P. damicornis* for each treatment.

Supplementary Table S4.7: PRIMER table showing SIMPER analysis between the OTUs from the t_{final} Heat Stress and t_{final} Control treatments with an average dissimilarity of 46.43%.

Supplementary Table S5.1: PRIMER table showing SIMPER analysis between the Control and Heat Stress treatments with an average dissimilarity of 41.78%.

Supplementary Table S5.2: PRIMER table showing SIMPER analysis between the t_{final} Heat Stress and t_0 treatments with an average dissimilarity of 56.02%.

Supplementary Table S5.3 PRIMER table showing SIMPER analysis between the thermal stress and control metagenomes with an average dissimilarity of 8.44%.

Supplementary Table S5.4 PRIMER table showing SIMPER analysis between the thermal stress and control metatranscriptomes with an average dissimilarity of 7.95%.

Abstract

Microorganisms live in tight associations with corals, but the ecological interactions and microbial functions and behaviours underpinning these relationships are not yet fully understood. The goal of this thesis is to define coral-microbe interactions by exploring how the composition, behaviour and function of microbial communities vary throughout a coral reef and how increasing sea water temperatures can affect coral-microbial relationships. As a first step to achieving this aim, In Chapter 1 we used metagenomics to characterise patterns in microbial composition and metabolic capacity across different niches, including coral-associated and non-coral associated microenvironments, on Heron Island, the Great Barrier Reef (GBR). We found that the composition and metabolic potential of coral reef bacteria is highly heterogeneous across a coral reef ecosystem, with a shift from an oligotroph-dominated community (e.g. SAR11, *Prochlorococcus, Synechococcus*) in the open water and sandy substrate niches, to a community characterised by an increased frequency of copiotrophic bacteria (e.g. *Vibrio, Pseudoalteromonas, Alteromonas*) in the coral seawater niches. Among the major functional patterns observed were significant increases in genes associated with bacterial motility and chemotaxis in samples associated with the surfaces of coral colonies. The observation of increased motility and chemotaxis near to coral surfaces is notable given previous evidence that these phenotypes may be involved in coral disease processes. The research presented in this chapter was published in Microbial Ecology (2014 67 (3): 540-552)

To investigate these patterns in chemotaxis further we next (Chapter 2) directly examined the potential ecological role of chemotaxis among coral-associated bacteria, by using laboratory based and *in situ* chemotaxis assays to test levels of chemotaxis among natural communities of coral reef microbes. We examined the behavioural responses towards several chemoattractants known to be released by corals and their symbiotic dinoflagelletes including amino acids, carbohydrates, ammonium chloride, and dimethylsulfonopropionate (DMSP). Using these approaches we found that bacteria associated with the surfaces of the corals

exhibited high levels of chemotaxis, particularly towards DMSP and several amino acids. Levels of chemotaxis by coral-associated bacteria were consistently higher than those demonstrated by non-coral associated bacteria. This work was published in the ISME Journal (doi: 10.1038/ismej.2014.261)

We next extended the *in situ* chemotaxis assays to examine the chemotactic behaviour of bacteria associated with other important coral reef organisms, sponges. These results redefine the sponge-symbiont acquisition paradigm whereby we show for that bacteria use chemotaxis to locate their sponge host on a coral reef. This work is in preparation for submission to the ISME Journal.

After defining some of the functions and behaviours involved in coral reef microbiology, we next examined how these processes may shift under changing environmental conditions, associated with climate change. To determine how environmental variability, specifically thermal stress, influences bacterial community composition, behaviour and metabolic capacity, manipulation experiments were conducted using the coral *Pocillopora damicornis*.

To investigate the dynamics of coral-associated vibrios under heat stress, in Chapter 4 we used Vibrio-specific amplicon sequencing approaches and qPCR to quantify shifts in the abundance and composition of natural populations of *Vibrio*, with a specific focus on the putative coral pathogen *V. coralliilyticus*. These experiments revealed that increasing seawater temperatures can favour the proliferation of potential coral pathogens among a natural mixed microbial community. This work has been published in Frontiers in Microbiology (6:432. doi: 10.3389/fmicb.2015.00432).

In Chapter 5, we decided to explore the entire coral-associated community by using metagenomics and metatranscriptomics to investigate how the phylogeny and function of coral associated microbes shift resulting from increasing seawater temperatures. We found a dramatic shift in the community from *Endozoicomonaceae* being dominant in the control corals, while there was an appearance of the vibrios under increasing sea water temperatures in line with our

findings from chapter 4. We also observed functional shifts that involved an up-regulation of chemotaxis and motility genes at higher temperatures and were shown to be affiliated with vibrios, a genus which contains several putative coral pathogens.

Taken together our data demonstrate that coral reef bacterial communities are highly dynamic and that key groups of copiotrophic bacteria have the capacity to use behaviours such as chemotaxis to use nutrient gradients to potentially locate and colonize benthic host animals including corals and sponges. Increasing seawater temperatures causes dramatic changes in the coral-associated bacterial community, allowing for the proliferation of potential coral pathogens and increased expression of behavioural phenotypes that may promote successful infection of corals.